phenotype is characterized by hearing and visual impairment, intractable epileptic encephalopathy, irritability, failure to thrive, progressive microcephaly, developmental stagnation, and reduced survival. GM3 synthase deficiency follows an autosomal recessive pattern with an estimated incidence of approximately 1 per 1,200 births in Old Order Amish communities of North America. No disease-modifying treatment is currently available. Here, we hypothesize that *ST3GAL5* replacement therapy targeted to the central nervous system (CNS) could drive ST3GAL5 expression in neurons and oligodendrocytes, restore the endogenous production and trafficking of cerebral gangliosides, and thereby rescue the severe neurodevelopmental phenotype. We first used lentivirus-mediated gene replacement to transfer human h*ST3GAL5* cDNA *in vitro*, which restored GM3 production in patient-derived fibroblasts. More importantly, h*ST3GAL5* replacement in patient iPSC-derived cortical neurons reconstituted GM3 as well as its major downstream a- and b-series brain gangliosides. These *in vitro* data indicate the strong therapeutic potential of human *ST3GAL5* cDNA gene replacement. Next, to prove our hypothesis *in vivo*, we packaged h*ST3GAL5* constructs into AAV9 capsids. To examine the safety and efficacy of transgene expression, we first delivered AAV vectors via intracerebroventricular (ICV) administration to wild type (WT) neonatal C57BL/6 mice. We observed robust transgene expression in CNS without short-term vector-associated toxicity. Furthermore, to assess the therapeutic efficacy of *ST3GAL5* gene replacement, we delivered AAV9-h*ST3GAL5* vectors in *St3gal5/B4galnt1* double knock-out (DKO) mice, which are unable to synthesize any cerebral gangliosides and mirror the phenotype of human GM3 synthase deficiency. Direct ICV treatment of neonatal DKO mice restored the production of GD1a, GD1b, and GT1b, improved physical growth, and extended median survival from 20 days to more than 5 months. To our surprise, administration of AAV9-h*ST3GAL5* via systemic injection triggered acute liver damage 2-days post injection that culminated in animal death. At the mRNA level, h*ST3GAL5* expression was 30-fold higher than normal in liver, which may be the cause of the liver damage. We therefore modified our vector design from a ubiquitous to neuronspecific promoter, which alleviated acute liver toxicity. In ongoing studies, we are further improving rAAV-based h*ST3GAL5* replacement vectors and testing their safety and efficacy in *St3gal5*-/- and *St3gal5B4galnt1* double knockout mice. [a]Co-corresponding authors

58. CRISPR/Cas9 Strategies to Treat Spinocerebellar Ataxia Type 1

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Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disease that causes progressive loss of motor coordination, respiratory issues and eventual death. SCA1 is caused by expansion of the polyglutamine repeat region in the *ATXN1* gene. Normal *ATXN1* alleles contain 6-42 CAG trinucleotide repeats with interspersed CAT nucleotides, while disease alleles have an uninterrupted CAG region with 39-100+ repeats. The mechanism of SCA1 pathogenesis is unknown; however, some features of the disease include neuronal degeneration and formation of toxic mutant ATXN1 (mATXN1) nuclear inclusions. Although mATXN1 is expressed ubiquitously, it affects primarily Purkinje cells (PCs). There are currently no treatment options for SCA1. We hypothesize that CRISPR-Cas9 editing of *ATXN1* will reduce mutant ATXN1 and be therapeutically beneficial. We designed two different strategies to reduce ATXN1; the first uses a single guide RNA (gRNA) to target near the exon-exon junction to induce nonsense mediated decay, while the second approach employs a dual guide system to delete the CAG repeat region. gRNAs were optimized in vitro, with each approach significantly reducing *ATXN1* expression. The single guide approach reduced *ATXN1* mRNA levels by 40-45% ($p \leq 0.02$) and protein by approximately 20% ($p \leq 0.01$) and the dual guide approach reduced levels of mRNA and protein levels by 70-75% ($p < 0.001$) and 45-65% ($p \leq 0.03$), respectively. For testing in vivo, SCA1 mice were crossed to *sp*Cas9 transgenic mice; SCA1 mice express human *ATXN1* with an expanded 82 CAG repeats in Purkinje cells, and show progressive motor deficits and neuropathology. Recombinant AAVs (rAAVs) expressing the optimized gRNAs (EE2 and EE4) from the exon-exon strategy were delivered directly to the deep cerebellar nuclei of 5-week-old SCA1/*sp*Cas9 mice for transduction of Purkinje cells. The EE2 gRNA reduced protein and mRNA levels by 55% ($p=0.05$) and 50% ($p=0.02$), respectively and EE4 decreased ATNX1 protein and mRNA levels by 10% ($p=0.9$) and 20% ($p=0.07$) compared to saline injected controls. The EE2 gRNA more effectively reduced ATXN1 levels, and studies are in progress to assess its impact on SCA1 mice phenotypes.

59. Rescue of Molecular and Motor Phenotypes in CGG Knock-In Mice with CRISPR Mediated Deletion of the Trinucleotide Repeat

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Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) is a neurodegenerative disorder that is caused by a premutation allele (55-200 CGG repeats) in the 5' untranslated region of the *FMR1* gene. Gene-editing using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) has previously been shown to delete the CGG repeats of *FMR1 in vitro*, but this has not yet been reported *in vivo*, nor the molecular and phenotypic effects of such a treatment strategy. We evaluated a Cas9 based therapy for its ability to correct *FMR1* trinucleotide repeats in the *Fmr1* CGG knock-in (KI) mouse with approximately 130 CGG repeats. These mice exhibit motor and memory impairments and are used to model FXTAS. Two guideRNAs (gRNAs) were used to target the CGG repeats for deletion upon Cas9 mediated cleavage. Dual AAV vectors containing Cas9 and the gRNAs were injected into mouse striatum and tissues harvested three weeks post injection. Isolated DNA

showed complete or partial deletion of the CGG repeats along with deletion of adjacent nucleotides upstream and downstream of the target site. The transcriptional start site and the start codon of *Fmr1*, elements required for functional gene expression, remained intact. Striatal expression of *Fmr1* in untreated KI mice had a 3-fold upregulation compared to WT littermates, consistent with the known upregulation of this transcript with CGG repeat expansion. However, KI mice treated with targeting gRNAs/ Cas9 had significantly lower *Fmr1* expression than untreated mice ($p < 0.0001$), with levels similar to WT. Treated and untreated mice from both genotypic groups had Fmrp levels that did not differ significantly from each other, suggesting the gene editing that corrected the regulation of *Fmr1* mRNA levels did not hinder synthesis of the encoded protein in *Fmr1* CGG knock-in mice. To investigate whether rescue of *Fmr1* transcriptional regulation would result in phenotypic rescue, neonatal mouse pups were injected at P0 to P1 bilaterally into the cerebral ventricles with the dual AAV vectors. Motor performance was evaluated at 12-15 weeks, 28-30 weeks and 52-54 weeks of age by accelerating rotarod. Motor impairment occurs in KI mice by 28 weeks, and results in quicker falls from the apparatus than is observed in WT littermates. Mice that were treated with the CRISPR constructs performed better than untreated knock-in mice, $p = 0.0041$ and were not statistically different their WT littermates at 28-30 weeks of age. At the late stage rotarod the *Fmr1* treated mice continued to perform similarly to the WT animals, but statistical differences were no longer observed between treatment groups. Additional analysis of behavioral and molecular data is ongoing.Our study is the first to demonstrate *in vivo* editing of expanded CGG repeats in *Fmr1* using CRISPR. Here we have shown that our guides enable safe excision, removing the expanded repeat, rescuing expression of *Fmr1* mRNA, and maintaining Fmrp synthesis. Importantly, we show *in vivo* CRISPR mediated editing of the *Fmr1* trinucleotide repeat has a therapeutic benefit, rescuing motor deficits present in aged KI mice. These results indicate that

CRISPR-mediated gene editing has the potential to ameliorate the degenerative pathology present in FXTAS and further development of this strategy for treatment of FXTAS and other Fragile X-associated disorders is necessary.

60. Transthyretin Gene Therapy as a Modulator of Alzheimer's Disease Progression

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Epidemiological studies and work in transgenic animals suggest that transthyretin (TTR) levels in plasma and/or CSF may modulate Alzheimer's disease (AD) presentation and/or progression. AD patients exhibit lower levels of TTR in CSF compared to agematched healthy individuals. Similarly, genetic reduction of TTR in mouse models of AD is associated with earlier onset and more severe phenotypes. The current study was designed to determine the potential of somatic AAV mediated TTR expression to modulate disease presentation in AD.We designed an AAV9 vector to express TTR specifically in liver and choroid plexus using a TTR promoter to drive transgene expression. Two-month-old male and female 5XFAD mice were injected systemically with 2E12 gc AAV9-TTRp-TTR^{T119M} (n=12M+12F), or PBS as controls (n=12M+12F). At nine months of age animals underwent neurobehavioral tests to assess cognitive function, and 3D isotropic T2-weighted MRI to evaluate differences in brain volume. Post-mortem assessments include histological evaluation of plaque burden, quantification of Abeta levels in different brain structures, quantification of neurofilament light-chain (NfL) in serum, among others still on-going.Cognitive function was assessed through measurement of working and spatial memory using forced and spontaneous alternation tasks in the Y-maze, and novel object recognition tests. We observed behavioral improvements in the females only, namely regarding in the percent time interacting with novel object. General locomotion and anxiety were evaluated using the open field test, where AAV treated females presented higher locomotor activity and increased exploratory behavior. Interestingly, no differences were observed in males. Surprisingly, MRI analysis revealed increased CSF volume in all treated animals compared to controls, with females showing larger differences.Histological analysis of plaque burden using 6E10 antibody staining showed a remarkable difference in the treated females in comparison to controls. Analysis of the males is still ongoing.Our initial results show that systemic delivery of an AAV9 vector that expresses TTR in appropriate tissues improves cognitive function, locomotor activity and anxiety symptoms in 5XFAD mice, but this benefit is more noticeable in females. The unexpected increase in CSF volume may be related to increased TTR production by the choroid plexus of AAV treated animals, and additional experiments are ongoing to determine the cause of this outcome.Overall, this new AAV vector represents a therapeutic platform to study biologically relevant questions about the role of TTR in AD.

61. CRISPR/Cas9-Mediated Excision of ALS/FTD-Causing Hexanucleotide Repeat Expansion in C9ORF72 Rescues Major Disease Mechanisms *In Vivo* and *In Vitro*

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A hexanucleotide repeat expansion (HRE) consisting of $GGGGCC_{24+}$ in the *C9ORF72* gene is the most common genetic cause of

amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Both are fatal neurodegenerative diseases with no current approved treatments that significantly slow disease progression or extend life expectancy. Several hypotheses have emerged to explain how this HRE causes neuronal death, including haploinsufficiency, sequestration of RNAbinding proteins in the nucleus, and toxic repeat-associated non-ATG (RAN) dipeptide production. In the present study we used a CRISPR/ Cas9 gene-editing approach to remove the HRE from the C9ORF72 genomic locus, designing guide RNAs (gRNAs) flanking the HRE, and delivered Cas9 and gRNAs via adeno-associated virus serotype 9 (AAV9) vectors. Here, we demonstrate successful excision of the HRE in C9ORF72 in primary cortical neurons and in the brains of three mouse models containing the C9ORF72 expanded HRE (ranging from 500-600 repeats) as well as in iPSC motor neurons and brain organoids (450 repeats). This resulted in a reduction of RNA foci and poly-dipeptides and a rescue of haploinsufficiency, the major hallmarks of C9-ALS/FTD. This work is, to our knowledge, the first to demonstrate an *in vivo* therapy that addresses both toxic gains-offunction conferred by to mutant RNAs and polydipeptides, but also haploinsufficiency, making this an extremely attractive therapeutic approach to these diseases.

62. C9ORF72 Variant-Specific RNA Interference Rescues C9-ALS/FTD Molecular Hallmarks *In Vivo* and *In Vitro*

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Amyotrophic lateral sclerosis (ALS) is a terminal neurodegenerative disease that affects upper and lower motor neurons, causing progressive muscle weakening and respiratory failure. A hexanucleotide repeat expansion (HRE) consisting of $GGGGCC_{23+}$ in the intronic region of C9ORF72, contributes to 40% of familial and 10% of total ALS cases. Up to 50% of patients with this expansion also develop frontotemporal dementia (FTD). The major proposed disease mechanisms behind this HRE include haploinsufficiency, RNA binding proteins sequestration in the nucleus, and toxic repeat-associated non-ATG (RAN) dipeptide production. Both C9ORF72 ALS and FTD are aggressive diseases with no treatments to significantly slow disease progression or extend life expectancy. We are developing two AAV-RNAi gene therapy approaches using artificial microRNAs packaged in AAVrh10 to: (1) specifically target C9ORF72 mRNA variants that contain the HRE (variant (V) 1 and V3) and preserve the most abundant variant, V2, which does not contain the HRE, to avoid haploinsufficiency; (2) specifically target the intron containing the HRE, thereby only targeting incorrectly spliced transcripts. Using approach (1) we significantly lowered levels of V1 in treated primary neurons of C9ORF72 BAC-transgenic mice expressing an expanded repeat (500) compared to untreated groups, while overall C9ORF72 levels remained unaffected. We next treated adult C9ORF72 BAC-transgenic mice through striatal injections and similarly reduced levels of V1 mRNA containing the HRE, but not in V2 in the striatum. This reduction of HRE-containing transcripts by artificial miRNAs resulted in a decrease of GP poly dipeptides and RNA foci. Our preliminary studies using approach (2) have demonstrated a large, significant decrease of intron-containing transcripts. Future experiments will test the efficacy of both therapeutic approaches *in vivo* and in patient-derived motor neurons to eliminate existing - and prevent the formation of - toxic dipeptides and RNA foci formation, to ultimately rescue motor neuron pathology in ALS and FTD.

63. Restoration of *Scn1a* Expression after Symptom Onset in a Novel Model of Dravet Syndrome Rescues Seizures and Behavioral Alterations

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Dravet syndrome is a severe epileptic encephalopathy that begins during the first year of life and leads to severe cognitive and social interaction deficits. It is mostly caused by heterozygous loss-of-function mutations in the SCN1A gene, which encodes for the alpha-subunit of the voltage-gated sodium channel (Nav1.1) and is responsible mainly of GABAergic interneuron excitability. While different therapies based on the upregulation of the healthy allele of the gene are being developed, the dynamics of reversibility of the pathology are still unclear. In fact, whether and to which extent the pathology is reversible after symptom onset and if it is sufficient to ensure physiological levels of Scn1a during a specific critical period of time are open questions in the field and their answers are required for proper development of effective therapies.We generated a novel *Scn1a* conditional knock-in mouse model (Scn1a floxSTOP) in which the endogenous Scn1a gene is silenced by the insertion of a floxed STOP cassette in an intron of Scn1a gene; upon Cre recombinase expression, the STOP cassette is removed, and the mutant allele can be reconstituted as a functional Scn1a allele. In this model we can reactivate the expression of Scn1a exactly in the neuronal subtypes in which it is expressed and at its physiological level. Those aspects are crucial to obtain a final answer on the reversibility of DS after symptom onset. In fact, for all the gene therapy approaches that are being explored in different laboratories, several factors converge on the final therapeutic efficacy: the real

ability of the strategy in boosting Scn1a gene expression at single cell level, the targeting of the correct neuronal subtype and the total number of cells reached by the treatment.We exploited this model to demonstrate that global brain re-expression of the Scn1a gene when symptoms are already developed (P30) led to a complete rescue of both spontaneous and thermic inducible seizures and amelioration of behavioral abnormalities characteristic of this model. We also highlighted dramatic gene expression alterations associated with astrogliosis and inflammation that, accordingly, were rescued by Scn1a gene expression normalization at P30. Moreover, employing a conditional knock-out mouse model of DS we reported that ensuring physiological levels of Scn1a during the critical period of

symptom appearance (until P30) is not sufficient to prevent the DS, conversely, mice start to die of SUDEP and develop spontaneous seizures. These results offer promising insights in the reversibility of DS and can help to accelerate therapeutic translation, providing important information on the timing for gene therapy delivery to Dravet patients.

AAV Biology, Engineering, Immunology and Animal Modeling

64. A Multi-Mechanistic Anti-Angiogenic AAV Gene Therapy Product Candidate, 4D-150, for the Treatment of Wet Age-Related Macular Degeneration (wAMD) and Diabetic Macular Edema (DME): Intravitreal Biodistribution, Transgene Expression, Safety and Efficacy in Non-Human Primates

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Introduction Major blinding conditions such as wAMD and DME are characterized by abnormal retinal angiogenesis resulting in leakage, hemorrhage and scarring with consequent vision loss. We have designed an intravitreally-delivered multi-mechanistic anti-angiogenic gene therapy (4D-150) which could prove an attractive alternative to currently-approved biologics by eliminating the need for frequent injections combined with the potential for improved efficacy. 4D-150 is comprised of the retina targeted and evolved intravitreal vector R100 and is engineered to deliver the aflibercept protein sequence (anti-VEGF A&B, Placental growth factor, PlGF) plus a second

sequence encoding ddRNAi targeting the angiogenic factor VEGF C. In our preliminary *in vitro* experiments, we have shown dose-related expression in human iPSC-derived retinal cells and demonstrated functional activity. **Methods** We assessed the in vivo efficacy of R100. anti-VEGF gene therapy (a 4D-150 prototype) in the validated NHP laser-induced choroidal neovascularization (CNV) model of wAMD across a broad range of therapeutically relevant doses. NHP were dosed IVT with the 4D-150 prototype and six weeks post-administration, underwent retinal laser photocoagulation to induce CNV lesions. After two and four weeks, the numbers of clinically relevant grade IV CNV lesions were assessed by fluorescein angiography. Anti-VEGF protein was assessed by ELISA over 12 months by serial in vivo aqueous fluid samples. We subsequently performed a study in nonhuman primates (NHP) to assess the safety of 4D-150 and to measure expression of the aflibercept protein (retina and ocular fluids) and expression of VEGFC RNAi in the retina. **Results** In the NHP CNV model, the 4D-150 prototype resulted in complete suppression of grade IV CNV lesions (0/72) in treated eyes compared to vehicle (19/72) at doses as low as 1E11vg/eye ($p<0.0001$). Measurable and dose-dependent anti-VEGF protein was detected in aqueous fluid as early as 14 days post dosing and sustained through 12 months. There were no ocular or systemic toxicities at any dose tested. Specifically, there was no evidence of chronic intraocular inflammation (uveitis). Single IVT administration of 4D-150 resulted in high levels of ocular and retinal aflibercept protein together with highly robust VEGF C RNAi expression, without evidence of toxicity. **Conclusion** 4D-150 is a multi-mechanistic antiangiogenic gene therapy that can be delivered by the simple and safe intravitreal route of administration. 4D-150 was designed with the goal of improved efficacy over single mechanism anti-angiogenic approaches by inhibiting multiple VEGF isoforms, as well as PlGF, within the retina. An intravitreal anti-VEGF prototype of 4D-150 resulted in efficacy and safety through 12 months in the NHP CNV model. Intravitreal 4D-150 resulted in sustained and high levels of functional aflibercept and anti-VEGF C RNAi. 4D-150 holds potential for the intravitreal treatment of wAMD and DME.

65. Evolving Synthetic AAV Variants for Genome Editing in Immune Cell Populations

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The immune system is an important target for genetic manipulation, with implications for cancer immunotherapy using T or NK cell populations, correction of monogenic blood disorders using hematopoietic stem cell populations or plasma cell-based therapies. A majority of approaches to achieve homology directed repair ex vivo are performed using AAV serotype 6 to deliver the single stranded DNA repair or donor template. However, significant room for improvement with regard to cellular targeting and efficiency of donor template or transgene delivery exists. To achieve such, we adopted our structuredriven evolution approach to engineer AAV capsid libraries that can transduce primary T-cells, B-cells, NK cells, monocytes/macrophages and hematopoietic stem cells of human and mouse origin. Upon cycling, newly evolved AAV variants were significantly enriched by

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> 1000 fold from the parental library. Highly conserved structural epitopes were mapped onto the capsid surface of lead capsid candidates, but amino acid residues were distinct for different cell lineages and with human vs murine origin as well. To assess the potential for improved transduction, we are evaluating lead AAV capsid variants in different human and mouse immune cell lineages. Notably, a human T cell evolved variant Ark312 and a mouse T cell variant Ark313 displayed 10-20 fold higher transduction efficiency in human T cells and in mouse T cells, 100-1000 fold improved transduction at low multiplicities of infection (MOI) showing a 100-1000 fold increased mean fluorescence index (MFI) with 40-fold increase in % transduced cells. Neither AAV variant infected immune cells from the other species, demonstrating host selectivity. When used to deliver an HDR/ donor template, Ark312 significantly increased knock-in frequency compared to AAV6 across a range of incubated viral titers. Remarkably, Ark313 enabled highly efficient HDR in primary mouse T cells with a knockin frequency reaching <25% at an MOI of 3e3 and above 50% at 1e5, whilst wtAAV6 afforded less than 10% at the highest MOI. These synthetic AAV variants and others under evaluation have the potential to enable greater ex vivo knockin efficiencies in a wide spectrum of immune cell-based therapies and ultimately help pave the way for immunoengineering in vivo.

66. Real Time Blood Brain Barrier Disruption in a Multi-Species Model

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Central nervous system (CNS) transduction by gene therapy is highly efficient upon local delivery. However, local CNS delivery is invasive and involve complex neurosurgical procedures that carry considerable risk for patients. Systemic delivery is the simplest route to achieve broad distribution of therapeutics in CNS, but they have to cross the blood brain barrier (BBB). Several approaches have been used to enhance the efficiency of AAV CNS gene transfer, including use of mannitol or focused ultrasound to temporarily disrupt the BBB, and the development of new AAV capsids. New AAV capsids developed by in vivo capsid evolution proved remarkably efficient for CNS gene delivery in mice, but the lack of superiority to AAV9 in non-human primates (NHP) raised questions about translatability and stopped further development as a new platform for human trials in neurological diseases. Methods that facilitate a transient opening of the BBB allowing for successful systemic delivery of AAV gene therapy, and the subsequent closing of the BBB, so that its function remains intact, are vital. Here we present the application of a BBB disruptive peptide, K16ApoE, that we show to achieve this transient BBB opening in multiple species.Initial studies were performed in young adult BALB/ cJ mice by co-injecting in the tail vein 5E11 vg AAV vector encoding Firefly luciferase (FLuc) with peptide, or injection of K16ApoE alone followed by AAV 30 min later. At 3-weeks post-injection, FLuc activity was measured in CNS and liver. In a second phase, we evaluated the BBB-disrupting properties of K16ApoE in other species by MRI using dynamic gadolinium (Gd) enhancement. Three different species were used: mouse, sheep and NHP, in a paired fashion, where one animal in each pair received a co-injection of 0.1 mmol/Kg Gd and K16ApoE and the other Gd only. The MR sequence was the same for all animals: baseline 3D T1W MPRAGE for structural imaging, dynamic 2D T1W imaging with a dynamic time of 20 sec for a total of 1 hour, and finally post injection repeat of the 3D T1W MPRAGE. Co-injection of AAV9-FLuc with K16ApoE enhanced FLuc activity in brain by ~100 fold compared to AAV9-FLuc alone, ~1,000 fold with AAVrh10 and ~10 fold with AAV2, a capsid that otherwise would not reach the brain when injected systemically. When we separated the administration of AAV9 and peptide, there were no differences in FLuc activity compared to control animals. For the second phase of the experiment, animals imaged during co-injection of Gd and K16ApoE showed higher levels of maximum signal enhancement within the brain compared to those that only received Gd (Figure 1). The contrast enhancement was observed at 2-3 min post injection and reached maximum after approximately 20 min, whereby the signal remained constant for the remainder of the imaging. In the animals that only received Gd, the signal was seen to increase immediately, consistent with contrast in the vascular system, and then return to baseline levels in less than 3 min. All animals recovered from anesthesia normally with no apparent side effects.K16ApoE peptide is able to transiently open the BBB and allows for transport of different AAV capsids as well as Gd over a span of less than 30 min, when compared to animals that did not receive peptide where there was little to no crossing of the BBB. The use of this peptide was well tolerated by all the species. The combination of systemic AAV gene therapy with a peptide that temporarily disrupts the BBB is a powerful approach to potentiate therapies for neurological diseases.

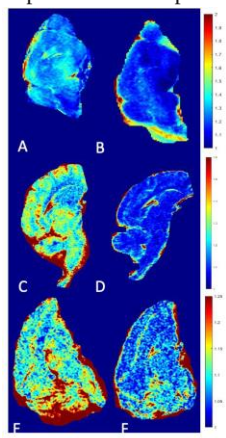

Figure 1:
Maximum enhancement maps for the dynamic T1W MRI. First column (A,C,E): Animals co-injected with gadolinium and K16ApoE. Second column (B,D,F): Animals injected with gadolinium alone. In the top row (A,B) shows mouse brains, the second row (C,D) shows sheep brains, and the final row (E,F) shows NHP brains. In all the species shown, the animals that received injection of gadolinium combined with peptide show greater levels of maximum enhancement over the brain parenchyma. In the animals that received gadolinium alone, only the choroid plexus (*) showed enhancement.

67. AAV2:2.retro-Mediated Delivery of Mutant Huntingtin throughout Cortico-Basal Ganglia Circuitry Leads to the Progressive Development of Motor and Cognitive Decline, along with Microstructural Changes in White and Gray Matter, in a Novel Rhesus Macaque Model of Huntington's Disease

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To create a nonhuman primate model of the neurodegenerative brain disorder, Huntington's disease (HD), we injected adult rhesus macaques into the caudate and putamen with a 1:1 mixture of AAV2 and AAV2.retro expressing a fragment of m*HTT* bearing 85 CAG repeats. Previous work by our lab shows that this injection strategy leads to the expression of mutant HTT protein, and the formation of hallmark HTT+ inclusions, throughout the striatum as well as dozens of cortical and subcortical brain structures due to the strong retrograde capability of AAV2.retro. Here, we queried the disruption of cortico-basal ganglia circuitry for 14-months post-delivery of this mHTT construct (HTT85Q, n=6), a control HTT construct bearing 10 CAG repeats (HTT10Q, n=6) or PBS buffer (n=5). We characterized the emergence of motor and cognitive phenotypes to link behavioral changes with disruptions in cortico-basal ganglia circuitry using multimodal neuroimaging techniques including diffusion tensor imaging (DTI) and T1/T2-weighted magnetic resonance imaging (MRI). To achieve this, we evaluated animals using complex behavioral tasks to assess fine motor coordination (Lifesaver Retrieval Task), gross motor function (NHP-specific neurological rating scale), working memory (Spatial Delayed Response) and object recognition (Delayed Non-Match to Sample) to complement our neuroimaging battery. Compared to buffer and HTT10Q treated controls, animals treated with AAV2:2retro-HTT85Q showed a progressive development of mild orofacial dyskinesia, aberrant forelimb posture, forelimb chorea, incoordination, hindlimb slowness (bradykinesia) and/or tremor, which were exacerbated by the dopamine receptor agonist apomorphine. Compared to baseline measures, control animals also performed better on the Lifesaver Retrieval Task than HTT85Q animals. Moreover, compared to controls, HTT85Q animals exhibited impaired spatial working memory, but preserved object recognition. Voxel-based DTI analysis revealed many white and gray matter regions with alterations in fractional anisotropy in HTT85Q animals, suggesting that mHTT expression resulted in microstructural changes throughout the cortico-basal ganglia circuit. Similar voxel-based approaches are currently being applied to the T1w/T2w images using tensor-based morphometry to address whether HTT85Q leads to localized changes in brain volume. Additional efforts are also underway using positron emission tomography (PET) imaging to explore perturbations in the dopamine system (F18-Fallypride) as well as regional changes in glucose metabolism (F18-FDG). These data

demonstrate the feasibility of generating AAV-based models of HD in nonhuman primates that exhibit the hallmark motor and cognitive behavioral phenotypes, as well as neuropathological manifestations, of HD. Using a combination of AAV2 and AAV2.retro allowed for the expression of mHTT throughout the cortico-basal ganglia circuit, versus just the striatum, leading to the creation a NHP model of this disease that more closely depicts the neuropathology observed in human HD patients. Therefore, our studies also set the stage for developing novel biomarkers of disease manifestation, as well as using this gene delivery approach to test promising therapeutics in our model.

68. Investigating Mechanisms of Variability of AAV5-hFVIII-SQ Expression in Mice

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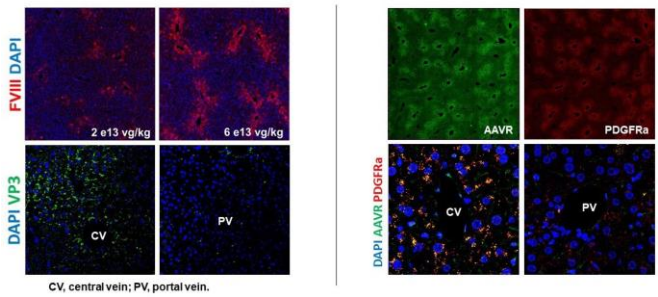
Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) is an investigational gene therapy under development for the treatment of severe hemophilia A. Significant intersubject variability has been observed in AAV5hFVIII-SQ gene expression outcomes across species and trials. We systematically investigated host factors in mice that may affect transgene expression at transduction, transcription, and protein translation/secretion. Male C57BL6 mice were administered a single vector dose ($1x10^{13}$ to $2x10^{14}$ vg/kg) intravenously. Levels of liver FVIII DNA, RNA, and protein; plasma FVIII protein; and various markers were analyzed. At doses of 1-$3x10^{13}$ vg/kg (producing therapeutic levels), significant correlation was noted between levels of liver vector DNA and FVIII protein ($r = 0.7312$, $P < 0.0001$), suggesting that hepatocyte transduction (from vector uptake, trafficking, genome processing to retention) may be an important contributing factor. At higher doses of vector ($\geq 6x10^{13}$ vg/kg), a strong correlation between levels of liver Grp78, a chaperone protein responsible for folding and secreting proteins, and plasma FVIII protein levels ($r = 0.7613$; $P < 0.0001$) was observed, suggesting individuals who have a greater intrinsic ability to fold FVIII protein may secrete higher levels of mature protein into circulation. While neutralizing AAV5 antibodies can inhibit transduction, other non-antibody soluble factors may also impact transduction. For example, levels of liver vector DNA positively correlated with predosing serum cholesterol ($r = 0.491$; $P = 0.0279$) and progesterone levels ($r = 0.496$; $P = 0.0261$). Next, we determined if abundance and expression patterns of AAV receptor (AAVR) and platelet-derived growth factor receptors (PDGFRa and b), known coreceptors of AAV5, correlated with AAV5 vector transduction. AAVR and PDGFRa expression exhibit a preferential pericentral vein pattern, similar to hepatocellular AAV5 capsid distribution and FVIII expression (**Figure**). There was a significant correlation between FVIII and PDGFRa expression ($r = 0.9546$; $P = 0.0115$) but not PDGFRb ($r = 0.7636$; $P = 0.1330$) or AAVR ($r = 0.1796$; $P = 0.7726$). Levels of DNA repair enzymes that facilitate the transformation of singlestranded vector DNA to circular genomes may also influence AAV vector transduction. The expression of one such exonuclease, Artemis (*DCLER1C*), was significantly correlated with AAV5-FVIII vector DNA levels ($r = 0.6822$; $P = 0.0052$). Next, we identified factors that may contribute to AAV-FVIII transcriptional variability. There was a significant correlation

between FVIII transcripts and mRNA levels of RNF121 (r = 0.7188; P = 0.003), Phf5A (r = 0.5970; P = 0.0154), and
HNF1α (a transcription factor that binds to the promoter of AAV5FVIII-SQ vector; r = 0.7158; P = 0.0054). Overall, we demonstrated that AAV5-FVIII-SQ intersubject variability may be driven by multiple contributing host-mediated mechanisms: transduction, transcription, and protein folding/secretion. Additional studies that further investigate the mechanistic drivers of AAV5 gene therapy variability are ongoing and may help identify predictive biomarkers of transgene expression and/or therapeutic approaches to decrease variability and optimize outcomes.

Figure. AAVR and PDGFRa expression in the liver of C57BL6 male mice shares a pericentral bias with FVIII transgene and vector capsid protein expression



CV, central vein; PV, portal vein.

69. Thermoresponsive Polymer-AAV Nanoparticle Vectors Improved Transgene Expression on Immunized Murine Model

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A great hurdle in adeno-associated virus (AAV) mediated gene therapy is pre-existing or re-administration-related humoral/cellular immune responses. Earlier, we developed an elastin-like polypeptide (ELP)based AAV delivery system and evaluated the performance in vitro in cell cultures. Our results showed that the virions encapsulated in the ELP nanoparticles can be shielded from neutralizing antibodies against AAV capsids. In this study, we have generated more ELP-AAV formulations (ELP $(KV_2F)_{64}$-AAV, ELP V_{60}-AAV, and PNIPAM-AAV) and screened them by neutralizing antibody assay. We found that ELP $(KV_2F)_{64}$ is the most effective polymer to protect AAV virion, and therefore we have used this ELP-AAV vector for further studies. Mass spectroscopy analysis indicated that the polymer was bound to residual K507, R566, and K649 of AAV VP3, and the molecular weight of ELP bonded VP3 was increased by 200 kDa. In vivo studies showed that the ELP-AAV2/9 nanoparticle vectors effectively transduced targeted tissue without tropism change compared to free AAV through subretinal injection, intramuscular injection, and retro-orbital injections. Histological examination indicated that no toxicity was observed on the major organs (kidney, liver, spleen, heart, lung, and brain) of mice injected with ELP-AAV2/9. The ELP-AAV2/9 vectors were also studied on the mice that were immunized with IVIg or prior AAV intramuscular injection via retro-orbital injection 2 hours after IVIg infusion or 2 weeks after intramuscular injection, respectively. Our results suggested that the ELP-AAV2/9 vectors significantly improved the reporter gene expression compared to free AAV and did not change the AAV tropism. Our ongoing studies are focusing on the delivery of using high-doses of ELP-AAV2/9 and alternative AAV serotypes. If successful, this method will create a novel strategy to potentially solve the immunogenic problem in AAV re-administration in clinic.

70. AAV Vector Dose Dependent Redundant and Non-Redundant Roles of TLR9 and IL1R Signaling in CD8⁺ T Cell Activation upon Muscle Gene Transfer

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Adeno-associated viral (AAV) vectors are evaluated in multiple clinical trials for the treatment of neuromuscular disorders. However, immune responses to the transgene product remains a concern. Viral vectors are initially sensed by the innate immune system, which shapes subsequent adaptive immune responses. Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns (PAMPs), while IL-1 receptors (IL-1Rs) intermediate downstream of pathways trigged by PAMPs or by tissue damage. They act as major sensors of pathogens for innate immune responses. Release of IL-1 cytokine serves as a damage signal to active IL-1R pathways upon infection and/or tissue injury. TLR9 is an endosomal DNA receptor that responds most potently to unmethylated CpG motifs as found in bacterial and viral DNA. Myd88 as a universal and essential adaptor is recruited when TLR9 and IL-1R signaling is activated. Our previous studies found that cross-priming of AAV capsid-specific $CD8^+$ T cells depended on TLR9-MyD88 pathway. Similarly, others documented TLR9dependent $CD8^+$ T cell responses against non-secreted transgene products such as LacZ and hemagglutinin upon muscle-directed AAV gene transfer. In our previous studies, we found that $CD8^+$ T cell responses to a secreted ovalbumin (ova) transgene product were substantially reduced (although not entirely eliminated) upon muscle gene transfer in TLR9-deficient mice [J Innate Immun. 7:302-14]. Here, we performed intramuscular injections with 2 doses of single-stranded ssAAV1-CMV-ova vectors ($2X10^{10}$ and $2X10^{11}$ vg) in wild-type (WT) C57BL/6 and innate sensing knockout (TLR9-/-, MYD88-/- and IL1R-/-) mice. Using MHC tetramer (H2-K^b -SIINFEKL), ova-specific $CD8^+$ T cell frequencies were monitored in peripheral blood for up to 6 weeks. As expected, transgene product-specific $CD8^+$ T cell responses were much reduced in MyD88-/- mice, in which 0.2% and 1.7% $tetramer^+$ of CD8 frequencies were found at low and high doses, respectively. To our surprise, TLR9-/- and IL-1R-/- mice only showed a substantially reduced response (1.2% and 0.1% $tetramer^+$ of CD8) at the low dose when compared to WT animals (12% $tetramer^+$ of CD8, $p<0.0001$, n=5/group), whereas $CD8^+$ T cell responses were similar in TLR9-/- and WT mice (16% and 15% $tetramer^+$ of CD8) and only slightly decrease in IL-1R-/- mice (11% $tetramer^+$ of CD8) at the high dose (n=5/group). To further investigate these findings, we prevented activation of IL-1R by pre-treatment with a combination of IL-1α and IL-1β antibodies followed by IM injections of $2X10^{11}$ vg of ssAAV1CMV-ova vector to TLR9-/- mice. As a result, substantially reduced $CD8^+$ T cell responses (4% $tetramer^+$ of CD8) were observed in these animals compared to TLR9-/- mice that

received isotype antibodies (13% tetramer$^+$ of CD8, p=0.003, n=5/group). Our data reveal that

sensing of the AAV genome by TLR9 and sensing of IL-1 release by IL-1R are both critical for the CD8$^+$ T cell response to the transgene product at lower vector doses. Thus, absence of one of the pathways dramatically impaired the response. However, TLR9 or IL-1R driven signaling pathways are sufficient to drive the response at higher vector doses, so that elimination of one of these now redundant pathways is not sufficient to blunt the response. Rather, both pathways need to be targeted at higher vector doses. We propose that use of CpG-depleted vectors (or TLR9 inhibitors) combined with IL-1R antagonist will be beneficial to prevent CD8+ T cell responses in AAV muscle gene transfer protocols that require high vector doses.

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71. Pre-Selected CAR $T_{N/SCM}$ Outperform CAR T_{BULK} in Driving Tumor Eradication in the Absence of Severe CRS and ICANS

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Capability of CAR T cells to expand and persist in patients emerged as a fundamental factor accounting for better outcome and durability of antitumor responses. These features inversely correlate with T-cell differentiation, suggesting that the enhanced T-cell fitness typical of early memory T cells may significantly improve CAR T cell therapeutic potential. Presently, however, whether pre-selecting specific memory T-cell subsets before manipulation would be really beneficial is still an open issue, especially as regard toxicity. Therefore, we deeply investigated the efficacy and safety profiles of CAR T cells generated from isolated naive/stem memory T cells ($T_{N/SCM}$), as compared to those derived from unselected T cells (T_{BULK}). As expected, CAR $T_{N/SCM}$ were less lytic than CAR T_{BULK} and produced lower amounts of pro-inflammatory cytokines when stimulated with CD19+ targets *in vitro*, even though displaying a similar proliferative capacity. When challenged against tumor cells in HSPC-humanized mice, limiting doses of CAR $T_{N/SCM}$ showed superior antitumor activity compared to CAR T_{BULK} and the unique ability to protect mice from leukemia rechallenge, together with higher *in vivo* expansion, persistence and

a better CAR T cell fitness. Indeed, as evaluated by BH-SNE algorithm, after leukemia encounter CAR $T_{N/SCM}$ were characterized by prevalence of early memory T-cell subsets, together with the expression of multiple activation markers and a limited enrichment of inhibitory receptors, as opposed to the more exhausted and terminally differentiated phenotype typical of CAR T_{BULK}. Notably, at limiting doses and low tumor burdens no cases of severe Cytokine Release Syndrome (sCRS) were reported. Conversely, when infusing high doses of CAR T cells in mice with

high tumor burdens, sCRS and Immune effector-Cell Associated Neurotoxicity Syndrome (ICANS) were only elicited by CAR T_{BULK}, with more than 50% of mice experiencing a drastic weight loss and increased serum elevation levels of IL-6 and SAA, culminating in the death of the treated mice. Moreover, multifocal brain hemorrhages were only found in the CAR T_{BULK} treated cohort, in contrast to the group infused with CAR $T_{N/SCM}$, in which only one mouse presented with a small hemorrhagic focus. Interestingly, similar results were obtained with CAR $T_{N/SCM}$ harboring a CD28 rather than a 4-1BB co-stimulatory molecule, indicating that CAR $T_{N/SCM}$ are intrinsically less prone than CAR T_{BULK} to trigger detrimental infusional toxicities, independently of the CAR design strategy. In conclusion, $T_{N/SCM}$ pre-selection during CAR T cell manufacturing allows for deeper and more durable antitumor responses in the absence of sCRS and ICANS, significantly widening the therapeutic index of current CAR T cell approaches.

72. CD5 CAR T-Cells Avoid Self-Elimination by Continuously Degrading CD5 Protein

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Chimeric antigen receptor (CAR) T-cells for T-cell malignancies have been clinically effective. However, CARs specific to T-cell antigens may also result in extensive fratricide of CAR T-cells, precluding their expansion. This constraint does not apply equally to all the T cell antigens potentially targetable by CAR-Ts. Thus, we have shown that T-cells expressing CD5-specific CARs produce limited initial fratricide and then become resistant to self-targeting despite having high CD5 expression prior to CAR transduction. Such minimal fratricide coincides with rapid and complete loss of detectable CD5 expression on the cell surface without affecting CD5 gene transcription, suggesting post-translational downmodulation of CD5 protein. However, the exact mechanisms underpinning antigen removal and the resulting resistance to fratricide in CD5 CAR T-cells remain unclear; an improved understanding of these processes may guide future efforts to target additional T cell antigens that are currently excluded from consideration due to excessive fratricide. Ligation of CD5 with monoclonal antibodies induces its capping and internalization. Using time-lapse microscopy in T-cells freshly

transduced with CD5 CAR, we observed rapid aggregation and internalization of surface CD5 from the cell membrane. Western Blot analyses revealed complete removal of the CD5 protein in CD5 CAR T-cells thus ruling out epitope masking or intracellular sequestration of the antigen. These results suggested CAR-mediated ligation of CD5 *in cis* triggers continuous internalization and degradation of CD5 protein in T-cells. Indeed, replacing endogenous CD5 protein with an engineered CD5 variant containing myc-/FLAG-tags on the N-/Ctermini resulted in complete loss of both tags in T-cells co-expressing CD5 CAR but not control CD19 CAR indicating the entire CD5 molecule is removed. Neither CAR nor CD5 signaling was required for antigen downmodulation as removal of intracellular signaling portions of each respective molecule did not ablate CD5 downregulation.

The process of CD5 removal can be initiated both *in cis* and *in trans*. CD5 CAR T-cells induced rapid *in trans* downmodulation of surface CD5 expression in both resting and activated primary T-cells upon short coculture with concurrent inter-cellular transfer of CD5 CAR molecules to target T-cells. Importantly, *in trans* removal of CD5 was observed in both normal and malignant T-cell lines suggesting this mechanism can limit availability of CD5 on target T-cells leading to resistance to cytotoxicity. Similarly to the *cis*-mechanism, complete removal of CD5 protein *in trans* was observed in target T-cells expressing dual-tagged [N-]myc/[C-] FLAG CD5 co-cultured with CD5 CAR Ts. However, in contrast to the *cis*-downmodulation, we detected robust release of soluble CD5 protein into culture supernatant during coculture of normal CD5+ T-cells with CD5 CAR T-cells, suggesting CD5 protein can also be shed or secreted from target cells upon contact with CD5 CAR T-cells. This study unravels a novel mechanism of fratricide evasion in T-cells expressing a T lineage antigen-specific CAR mediated by continuous removal of target antigen. Furthermore, rapid downmodulation of CD5 on normal and malignant T-cells may contribute to their resistance to CD5-directed cytolysis. These results are supported by clinical observations from an ongoing Phase I clinical study in which CD5 CAR T-cells could produce complete regression of recalcitrant T-cell tumors without fully ablating the endogenous T-cell compartment. Understanding the mechanisms of fratricide resistance can inform design of other T lineage-specific CARs and improve outcomes in patients with T-cell malignancies.

73. Abstract Withdrawn

74. Investigating the Therapeutic Efficacy of Disruption of Cell Intrinsic Checkpoint Regulator CTLA-4 in Chimeric Antigen Receptor T Cells

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Cancer immunotherapy is a rapidly growing field that has led to multiple successful treatment strategies including monoclonal antibodies (MAb), inhibitory receptor (IR) blockade, and adoptive cell transfer (ACT). ACT modified to express chimeric antigen receptors (CARs) can redirect T cells to tumor-associated antigens and has shown impressive clinical efficacy in patients with refractory leukemia and lymphoma. 90% of pediatric patients with acute lymphoblastic leukemia (ALL) respond to CD19 CAR T cell therapy (CART19), whereas only 26-35% of patients with chronic lymphocytic leukemia (CLL) show complete responses (CR). It is unclear why responses are less frequent in CLL compared to ALL. The heavy pre-treatment received by CLL patients likely contributes to lower response rates with T cell therapy, and disease progression is worsened due to profound T cell defects characterized by elevated expression of IRs such as PD-1 and CTLA-4. We hypothesized that disruption of CTLA4 would improve CAR T cell efficacy in CLL based on numerous observations. Fraietta et. al. assessed CLL patient apheresis samples and CART19 products to show that CR was associated with elevated levels of CD27+PD1-CD8+ T memory cells whereas the non-responders (NR) showed an exhausted phenotype with high levels of multiple IRs. We studied 14 patients with advanced, heavily pretreated CLL who received at least one dose of CART19. Patients with CRs exhibited high *in vivo* expansion and persistence of infused CAR T cells, as opposed to NR's. Importantly, at peak levels of *in vivo* CAR expansion, NR's had elevated levels of CTLA-4 expression which correlated with poor CLL patient responses to CART19 therapy. Additionally, multiple studies show CTLA4 is amongst the top 20 differentially upregulated genes in dysfunctional CD8+ T cells from various tumors such as hepatocellular carcinoma, melanoma, non-small cell lung cancer, and pancreatic adenocarcinoma. In summary, these data suggest that eliminating CTLA-4 mediated T cell inhibition can be clinically beneficial. We, therefore, performed disruption of CTLA4 using CRISPR technology in primary human T cells from healthy donors. Our data demonstrate that the knockout (KO) of CTLA-4 leads to maintenance of surface CAR expression and higher tumor clearance in a chronic re-stimulation model using CART19 cells against NALM6 tumor cells. In xenograft models of ALL, KO of CTLA-4 increases the anti-tumor efficacy of CART19 cells. We then performed CTLA4 disruption in CLL patient T cells who had not responded to CAR T cell therapy to determine whether dysfunctional CAR T products from CLL patients can be invigorated by CTLA-4 KO. In the chronic re-stimulation model of NALM6, CTLA-4 KO CAR T cells from CLL patients maintained CAR expression and lower tumor burden, relative to subject-matched unedited CAR T cells. Thus, disruption of CTLA4 in CD19 CAR T cells from NR CLL patient cells endows them with superior anti-tumor efficacy, suggesting that CTLA4 disrupted CAR T cell products may enhance the success rate of CAR T cell therapy for CLL patients. This technology can be feasibly expanded to other tumor indications and

increase the overall efficacy of CAR T cells. These IND-enabling studies will support the translation of this therapy to the clinic.

CAR Modified Cellular Therapies

75. Non-Human Primate Derived CD20 CAR T Cells Elicit a Bystander Effect on CD8 but Not CD4 CAR- T Cells

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The recent clinical successes of CAR T cell (CAR T) therapy are reshaping the field of hematology-oncology. However, despite the significant response to CD19 CAR T therapy in B-ALL, 10-20% of patients fail to enter remission and, perhaps more importantly, 30-50% of patients who achieve remission relapse within a year. Given these results, it is critical to understand not only the drivers of CAR T effector functions but also the mechanisms by which one can induce a tumoricidal bystander effect in surrounding effector cells. Performing detailed analysis of CD19 CAR Ts in human subjects is challenging mostly due to the limited sample volume available. To address this shortcoming, we have now utilized a clinically relevant Non-Human Primate (NHP) CD20 CAR T model to identify mechanisms of the CAR T specific immune response. For this we adoptively transferred CD20 CAR Ts to lymphodepleted rhesus macaques. CAR T expansion resulted in B cell ablation, associated with clinical symptoms of cytokine release syndrome. CAR T persisted an average of 4 weeks, at which point loss of CAR T was followed by B cell recovery. Flow analysis of the CAR- T population showed only minimal activation status of CD4 CAR- Ts and expansion of CAR Ts was more prominent in CD8 T cells. To further reveal the bystander effect of CAR- Ts we performed single cell sequencing analysis of sorted CAR+ and CAR- Ts from the product and at time of maximum proliferation. As expected, CAR- and CAR+ Ts in the infusion product showed similar transcriptomic profiles, however, state of activation differed significantly between CD4 CAR- and CD4 CAR+ Ts at peak of expansion (Wilcoxon test, p-value <0.001). While after infusion CD4 CAR- Ts reverted to memory- like state, CD4 CAR+ Ts maintained an effector state. In contrast, CD8 CAR+ and CD8 CAR- Ts both displayed a very similar effector pattern in the product and at maximum expansion, suggesting, that CAR- CD8 Ts, in contrast to CAR- CD4 Ts, are subject to a significant bystander effect. This is also reflected in the analysis of differentially expressed genes: at time of peak expansion we detected > 200 differentially expressed genes in between CD4 CAR- and (n=832) CAR+ Ts (n=594). In contrast differential gene expression analysis of CD8 CAR+ (n=163) vs CAR- Ts (n=556) at peak expansion did not demonstrate significant differences. Ingenuity Pathway Analysis comparing differentially expressed genes in CD8 CAR+ and CAR- Ts in peak expansion vs infusion product suggests a role of mir155-

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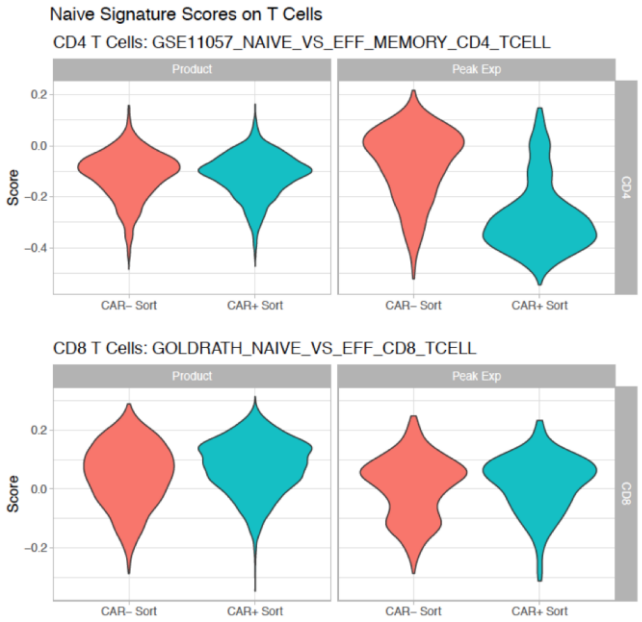
driven pathways in the CD8 CAR- T bystander effect.In summary these data show for the first time that a CAR induced bystander effect is predominantly elicited in CD8 CAR- Ts. Further dataset analyses as TCR based T cell tracking are underway to uncover additional mechanism of the immune regulation of CAR Ts in the NHP model.



The signature score for each cell was calculated using the software package VISION, which treats cell signatures from MSigDB as scores (Upregulated = +1,Downregulated = -1), and multiplies these scores by the normalized expression counts in each cell. Results are displayed as violin plots, separated by CAR+/CAR- status, and timepoint.

76. Enhanced Generation of T-Cell Derived Naïve Pluripotent Cells as a Renewable Cell Source for the Mass Manufacture of Off-the-Shelf CAR T Cell Therapies

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Induced pluripotent stem cells (iPSCs) are a promising renewable cell source for the mass manufacture of uniform chimeric antigen receptor (CAR) T cell products that can be banked and validated in advance to relieve major manufacturing cost and logistical obstacles. iPSCs can be generated from a variety of somatic cell types (commonly fibroblasts or CD34+ cells) but T cells are an ideal starting material for the derivation and engineering applications